Abstract

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Title of diploma thesis: Complementation of *Francisella tularensis dsbA* mutant strain and analysis of potential binding partners of DsbA protein

Conserved hypothetical lipoprotein FTT1103 is a virulence factor of gram-negative intracellular bacterium *F. tularensis*. This protein shares homology with proteins of disulfide oxidoreductase DsbA family. These proteins catalyse formation of disulfide bonds, which are essential for forming proteins conformation, for activity and stability of proteins. The aim of this study was to identify binding partners of lipoprotein FTT1103, commonly known as DsbA. The knowledge of these partners could help us in understanding a role of lipoprotein DsbA in virulence *F. tularensis*.

We prepared fused *ftt1103* gene with sequence coding FLAG® tag and cloned this fused gene into plasmid vector which can be replicated in *F. tularensis*. We used electroporation for introducing plasmid vector into the *in frame* deletion mutant *F. tularensis* subsp. *holarctica* strain FSC200/Δ1103 thereby we complemented this deletion mutant *in trans*. Imunoprecipitation by ANTI-FLAG® M2 Affinity Gel was performed to isolate protein DsbA with FLAG® tag and its binding partners from the complemented strain. The obtained proteins were cleaved on peptides by trypsin. The peptides were analysed by tandem mass spectrometry. The acquired data were scored against *F. tularensis* subsp. *holarctica* LVS database.